Section A-Research paper
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Profiles of Bacterial Isolates Exhibiting Antibiotic Resistance from Hotspot Sites in Khammam City, Telangana

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KEY WORDS:
AMR - Anti microbial resistance, AST - Antimicrobial susceptibility testing, CLSI - Clinical and Laboratory Standards Institute, MARI - Multiple antibiotic resistance index.

ABSTRACT:
Antibiotic resistance has escalated into a serious worldwide concern. Antibiotic-resistant infections and microorganisms in the environment are heavily influenced by hotspot habitats. This study used a selective growth medium to isolate, characterize and profile various antibiotic-resistant bacteria found in hotspot environments of the Khammam district. The selective growth medium contained bacterial isolates at various concentrations of Ampicillin, Amoxicillin clavulanate, Cefuroxime, Ceftriaxone, Imipenem, Gentamycin, Nalidixic Acid, Ciprofloxacin, Erythromycin and Tetracycline.

A serious worldwide catastrophe has emerged due to antibiotic resistance. The spread of diseases and microorganisms that are resistant to antibiotics is greatly aided by hotspot conditions. This study used a selective growth medium to isolate and characterize various antibiotic-resistant bacteria found in hotspot environments of the Khammam district. These bacteria were resistant to Ampicillin, Amoxicillin clavulanate, Cefuroxime, Ceftriaxone, Imipenem, Gentamycin, Nalidixic Acid, Ciprofloxacin, Erythromycin and Tetracycline.

Bacterial and biochemical studies have identified *Escherichia coli*, *Pseudomonas*, *Klebsiella*, *Proteus*,*Staphylococcus* and *Acinetobacter* are being the resistant species. In accordance with this study, hospital hotspots are abundant of these antibiotic resistant bacteria, posing a health risk to people. *Escherichia coli* and *Pseudomonas* are the most common bacterial isolates, as per this study's findings.

1. INTRODUCTION:

Wastage from hotspot habitats are reservoirs for an assortment of microorganisms and may represent an imminent danger to human health and represent a significant obstacle for contemporary medicine. Unregulated microbial resistance drives up healthcare costs and raises the likelihood of

fatal infections, which eventually results in worsened ailments that result in illnesses and death of a person (6).Antibiotic overuse is responsible for an upsurge in antimicrobial resistance. At first, it was thought that bacteria resistant to antibiotics could only be discovered in a medical facility, but it has recently been suggested that they could potentially found in an array of various circumstances.
Even though hospitals are the primary suppliers of bacteria with antibiotic resistance, these germs have a variety of routes via which they might spread to the general population through waste disposal (3). According to a number of publications, gram negative isolates from pathogens are more likely to be found in waste materials and to exhibit multidrug resistance. This antimicrobial resistance can spread to the digestive systems of both humans and animals. The major threat currently facing everyone is the development of resistant environmental microorganisms that are evolving into illnesses transmissible to humans.
Given the aforementioned elements, it was clear that drug resistance currently poses a serious threat to people's lives. The primary goal of the current study is to investigate the resistance profile of bacterial isolates from various hotspot habitats in accordance with the aforementioned criteria.

2. MATERIALS AND METHODS:

2.1. Materials:

This experiment employed selective culture media from Hi-medium, India, including nutrient agar media (NA), nutrient broth media, MacConkey agar, EMB agar, and Agar with Cetrimide. To meet the supplier's specifications, all media were prepared.

2.2. Antibiotic stock solution preparation:

In a 250 ml volumetric flask, 300 mg each of the following antibiotics were dissolved: Ampicillin(AMP), Amoxicillin clavulanate(AMC), Cefuroxime(CFM), Ceftriaxone(CTR), Imipenem(IMI), Gentamycin(GEN), Nalidixic Acid(NAL), Ciprofloxacin(CIP), Erythromycin(EN) and Tetracycline(TE). Within five minutes, the antibiotic had fully dissolved. In accordance with the manufacturer's instructions, the volumetric flask was sealed and kept at room temperature.

2.3. Sample collection:

Samples are taken from locations that are considered to be hot areas, such as city halls, factories, and medical facilities (7). Using sanitized sample bottles, 48 samples (23 medical waste and waste water, 18 municipal waste and waste water, and 7 industrial waste and waste water) were obtained from 10 locations in Khammam City. Swab samples, soil samples, and water samples were taken from various locations, and after being brought to the lab, these samples were processed according to standard protocol for bacterial isolation and susceptibility testing.

2.4. Bacterial Isolates: Isolation and Identification:

All bacteriological evaluations were performed in triplicate, and colonies were picked and purified via successive subcultures using specific media such as Mannitol salt agar for *S. aureus*, EMB for *E. coli*, cetrimide agar for *Pseudomonas*, Blood agar for *Proteus*, and Macconkey for identifying *Acinetobacter* isolates. Following defined protocols and comparing the isolates' characterization with Bergey's Manual of Determinative Bacteriology, bacteria were identified based on their morphology, Gram staining, cultural, and biochemical tests (Methyl Red/Voges-Proskauer, catalase,

indole, urease, citrate utilization, triple sugar iron, Sulfide, Indole, Motility (SIM), oxidase, and carbohydrate fermentation).

2.5. Bacterial isolation with regard to antibiotic resistance:

In the liquid broth medium, bacteria from various waste kinds were cultivated. However in this culture, every form of bacterium and microbial community were visible. As an initial culture, this culture was employed. Ampicillin, Amoxicillin clavulanate, Cefuroxime, Ceftriaxone, Imipenem, Gentamycin, Nalidixic Acid, Ciprofloxacin, Erythromycin and Tetracycline were diluted to 12 ppm, 6 ppm, 3 ppm, 1.5 ppm, 0.75 ppm, and 0.375 ppm in nutrient broth medium, respectively. The precise quantity of antibiotics with 100 ml of nutritional agar solution , was placed into a sterile 250 ml conical flask. After that, 1 cc of bacterial starter was added to the conical flask and aseptically grown there for 48 hours at 37°C. The resistant bacteria were found to be in a primitive culture. Then bacteria in 20µl aliquots were inoculated onto petridishes containing 10 ml growth medium. For an extended period of 24 hours at 37 °C, the petridishes were inverted in the incubator for growth. The procedure was carried out under sterile conditions.

2.5. Antimicrobial Susceptibility Testing:

Using standardized disc diffusion, the susceptibility of bacterial isolates to each of these antibiotics was evaluated (2). In order to examine the zone of inhibition, antibiotic discs were carefully placed on the surface of nutritional media that had previously been inoculated with the isolates. The measured zones of inhibition were classified as resistance, intermediate and sensitive upon examining the results (4).

Another approach for determining minimum inhibitory concentrations (MICs) of antimicrobial drugs is the dilution method (1). In this, the microbes' capability for promoting growth on agar is examined. This comprises reports of several antibiotics that exhibit resistance, sensitivity, and sample inhibition (5).

At each step of the investigation, the standard procedure was followed to ensure the reliability of the data. Before starting the procedure, the instruments' working was tested. Following the manufacturer's instructions, the quality of the media, reagents, stains, and antibiotic discs was assured. The disc diffusion test and biochemical assays were carried out in accordance with CLSI guidelines using reference strains of E. coli (ATCC 25922) and S. *aureus* (ATCC 25923) as controls. Data analysis was also done by using descriptive statistics. MARI values were also calculated by using the formula of no. of antibiotics showing resistance to the total number of antibiotics tested.

3. RESULTS AND DISCUSSIONS:

3.1. Bacterial isolates isolated from each sample point, in number:

A total of 48 swab, soil and water samples were processed for the presence of drug-resistant bacterial pathogens. Of these samples, 100% of the samples were positive to one or more isolates. Out of these 23 positive samples from medical waste, 16 samples from Municipal waste and 3 samples from Industrial waste were recovered. Among the total samples, 83 bacterial isolates were

recovered. Among them, 48(57.8) were from the medical waste, 26(31.3) were from municipal waste and 9(10.8) from industrial waste.

Table 1. **Bacterial Isolates isolated from each sample point, in number:**

Type of Sample	Total samples N (%)	Poitive samples N (%)
Medical waste	23 (46.9)	23(46.9)
Municipal waste	18 (36.7)	16(32.7)
Industrial	07 (14.3)	03(6.1)
Total	48 (100)	42(85.7)

Table 2. Number of different bacterial isolates isolated at each sampling point.

Name of Bacterial Isolates	No. of Isolates In Medical Wastage	No. of Isolates in Industrial Wastage	No. of Isolates in Municipal Wastage	Total Number of Isolates
E.coli	18(52.9)	02(5.90)	14(41.2)	34(41.0)
Pseudomonas	12(50.0)	03(12.5)	09(37.5)	24(29.0)
Klebsiella	09(75.0)	02(16.7)	01(8.3)	12(14.5)
Staphylococus	06(66.7)	02(22.2)	01(11.1)	09(10.8)
Proteus	02(66.7)	0(0)	01(33.3)	03(3.60)
Acinetobacter	01(100)	0(0)	0(0)	01(1.20)
Total	48(57.8)	09(10.8)	26(31.3)	83(100)

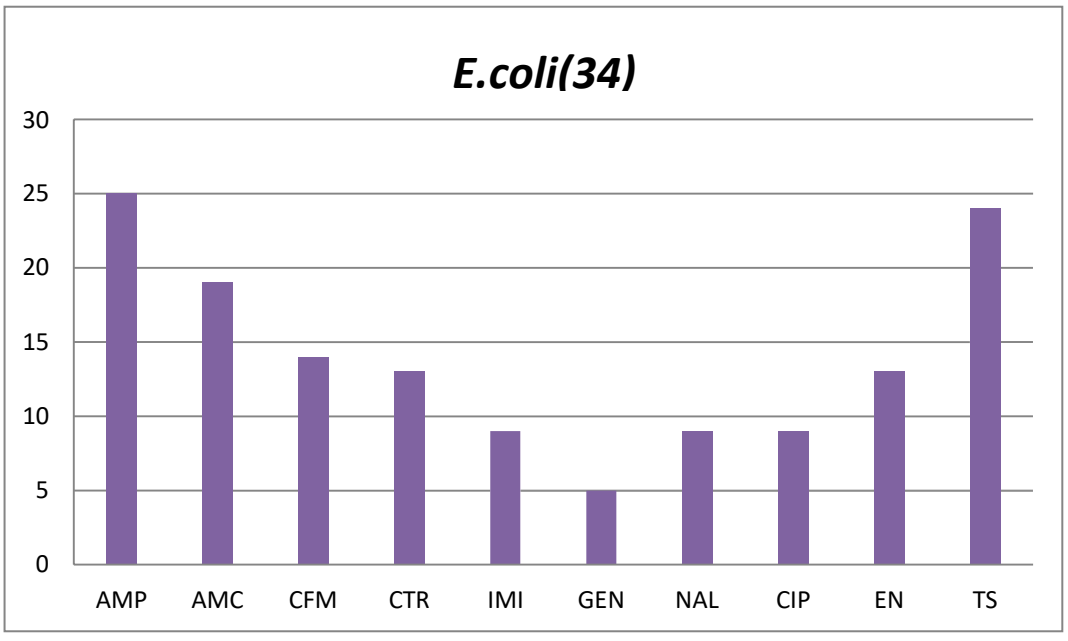

Fig1 *: E.coli* showing resistance to antibiotics

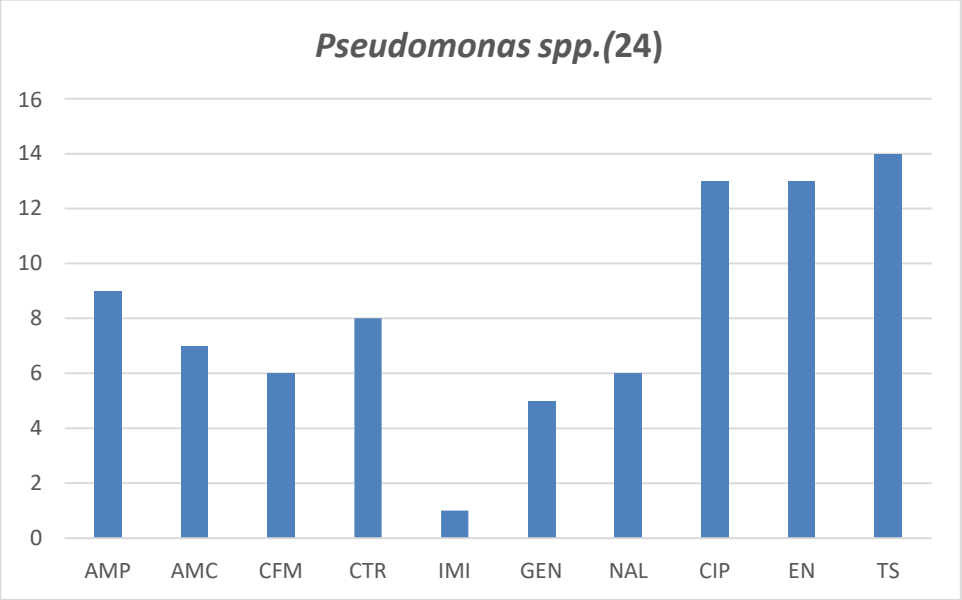

Fig2: *Pseudomonas spp.* showing resistance to antibiotics

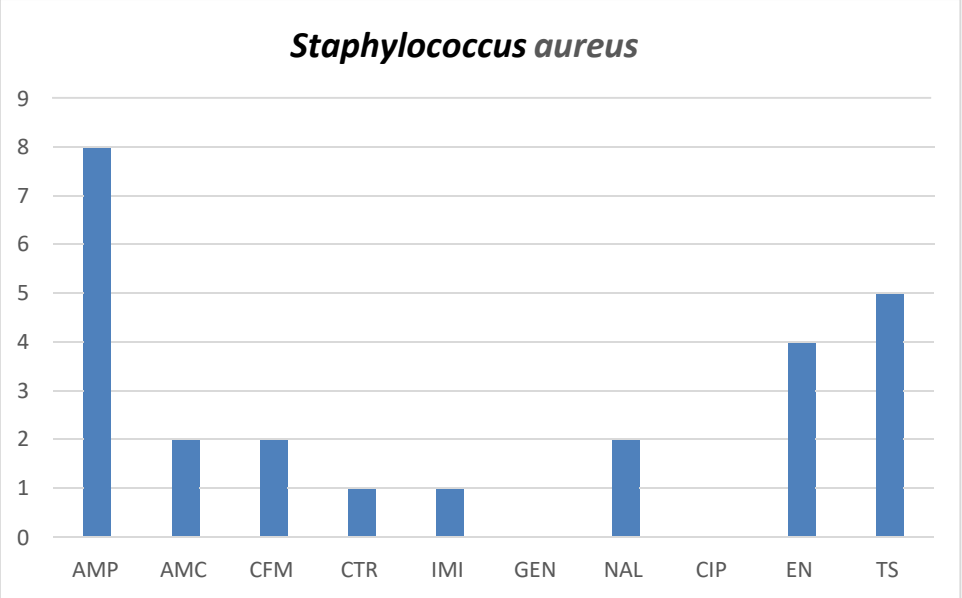

Fig 3: *Staphylococcus spp*.showing resistance to antibiotics.

Staphylococcus

3.2. Profiles of Bacteria exhibiting Antibiotic Resistance isolated from Hotspot Sites in Khammam City, Telangana:

As shown in Table -3 among all isolates of bacteria *Staphylococcus aureus* showed the highest pattern of resistance to ampicillin 8(88.9%) except considering *Acinetobacter*. In all isolates,The highest resistance was exhibited by Tetracycline Antibiotic. The highest resistance recorded for *E.coli* 24 (70.6%), *Pseudomonas spp.* 14 (58.3%), *Klebsiella spp.* against Tetracycline 9 (75%).

Among these all isolates, the highest resistance bacterial isolates were *E.coli* (34). In total, the bacterial isolates obtained from medical wastage, municipal wastage and industrial wastage were mostly resistant to ampicillin 45 (93.8%), 21 (80.8%) and 7 (77.8%) respectively.

Table 3: Profiles of Bacteria exhibiting Antibiotic Resistance against tested antibiotics from Hotspot Sites in Khammam City, Telangana;

No.of isolates of bacteria(N)	**AMP N (%)**	**AMC N (%)**	**CFM N (%)**	**CTR N (%)**	**IMI N (%)**	**GEN N (%)**	**NAL N (%)**	**CIP N (%)**	**EN N (%)**	**TE N (%)**	**MARI**
E.coli(34)	23(67.6)	19(55.9)	14(41.1)	13(38.2)	9(26.5)	5(14.7)	9(26.5)	9(26.5)	13(38.2)	24(70.6)	1.0
Pseudomonas spp.(24)	11(45.8)	7(29.2)	6(25)	8(33.3)	1(4.2)	5(20.8)	6(25)	13(54.1)	13(54.1)	14(58.3)	1.0
Klebsiella spp.(12)	6(50)	3(25)	3(25)	2(16.7)	3(25)	7(58.3)	2(16.7)	7(58.3)	7(58.3)	9(75)	1.0
Proteus spp.(3)	2(66.6)	1(33.3)	1(33.3)	1(33.3)	0(0)	0(0)	1(33.3)	1(33.3)	1(33.3)	0(0)	0.7
Staphylococus aureus(9)	8(88.9)	2(22.2)	2(22.2)	1(11.1)	1(11.1)	0(0)	2(22.2)	0(0)	4(44.4)	5(55.5)	0.8
Acinetobacter spp).(1)	1(100)	1(100)	1(100)	1(100)	0(0)	0(0)	0(0)	1(100)	0(0)	0(0)	0.5
Total(83)	51(61.4)	33(39.8)	27(32.5)	26(31.3)	14(16.9)	17(20.5)	20(24.1)	31(37.3)	38(45.8)	52(62.7)	

Table 4: Profiles of Bacterial Isolates exhibiting Antibiotic Resistance Isolated From Hotspot Sites in Khammam City, Telangana.

No.of isolates of bacteria(N)	**AMP N (%)**	**AMC N (%)**	**CFM N (%)**	**CTR N (%)**	**IMI N (%)**	**GEN N (%)**	**NAL N (%)**	**CIP N (%)**	**EN N (%)**	**TS N (%)**
Medical wastage (n=48)	45(93.8)	37(77.1)	33(68.8)	33(68.8)	19(39.6)	21(43.8)	24(50)	29(60.4)	38(79.2)	31(64.6)
Municipal astage(n=26)	21(80.8)	22(84.6)	18(69.2)	17(65.4)	12(46.2)	14(53.8)	13(50)	17(65.4)	16(61.5)	18(69.2)
Industrial(n=9) wastage	7(77.8)	3(33.3)	3(33.3)	3(33.3)	0(0)	0(0)	2(22.2)	4(44.4)	4(44.4)	3(33.30
Total(83)	73(88))	62(74.7)	54(65.1)	53(57)	31(37.3)	35(42.2)	39(41.9)	50(60.2)	58(70)	52(62.7)

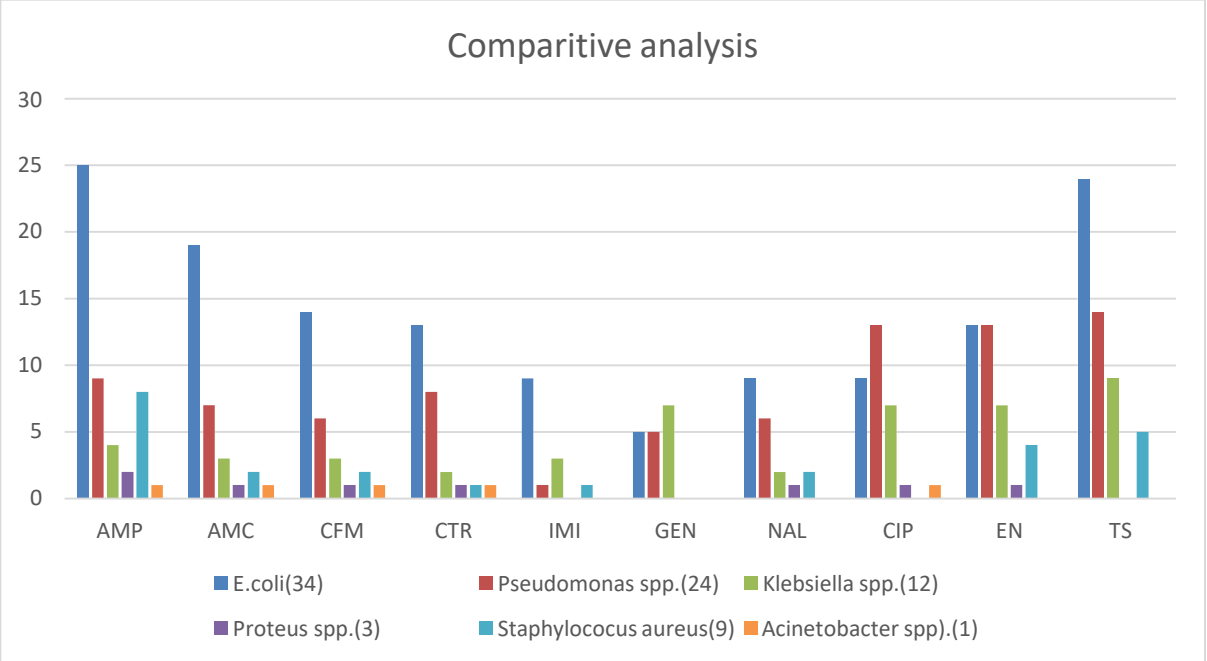

Fig4: Resistance profiles of each antibiotic in comparison.

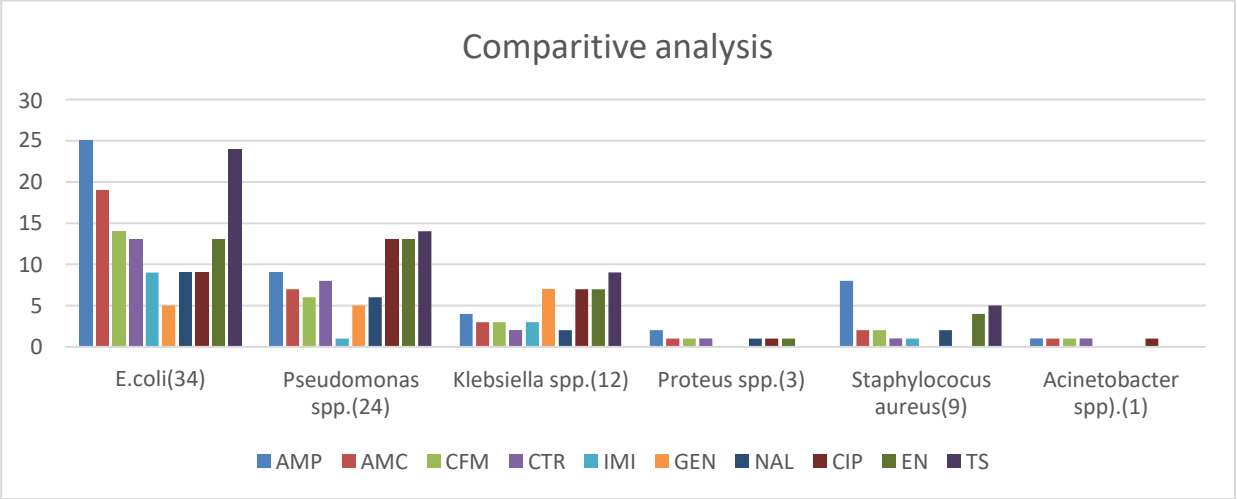

Fig5: Resistance profiles of each bacterial isolates in comparison.

The findings in this study reveal the high resistancerates are obtained for isolates of *Escherichia coli*, *Pseudomonas spp.* and *Klebsiella* spp. Tetracycline encountered a significant resistance, comparable to that in Ampicillin in *E. coli*, *Pseudomonas spp*. and *Klebsiella spp*. This study also showed that *E. coli*, *Pseudomonas* spp. and *Klebsiella* spp had the highest multidrug resistance, with a MARI score of 1.00, and that all isolates tested positive for resistance to all 10 drugs.The MAR index of *Proteus spp*., *S.aureus* and *Acinetobacter spp*.were 0.7, 0.8 and 0.5 respectively.

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Acinetobacter spp. were showing lower resistance due to less no .of isolates. High number of isolates of *Acinetobacter* are required to find out the percentage profiles. Multiple drug resistance was common in gram negative isolates. These findings were also showing that resistant rates vary from one habitat to other.The present study also showed that isolated bacteria were more resistant to commonly employed antibiotics such as Cefuroxime, Ampicillin and Amoxicillin. From the results it is cleared that hotspot habitats are full of drug resistance pathogens that are mainly resistant against commonly used antibiotics. It is also revealing that untreated environments lead to increase of pathogenic bacteria.

5. CONCLUSION:

These conclusions have an impact on the selection of antibiotics for infection management in hospitals and for empirical treatment of infections. To prevent the spread of these infections, effective control measures must be in place. To develop preventative and alternative treatments, like as vaccinations and antibiotics, for these organisms, more research should be done to analyze and identify the genes responsible for resistance and/or virulence.

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